0200

OIPE #4

RAW SEQUENCE LISTING

DATE: 07/05/2000

PATENT APPLICATION: US/09/598,982

TIME: 18:43:07

Input Set : A:\Tryptase.app

Output Set: N:\CRF3\07052000\1598982.raw

ENTERED

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3 <110> APPLICANT: NILES, ANDREW L
              MAFFITT, MARK A
                HAAK-FRENDSCHO, MARY
         <120> TITLE OF INVENTION: RECOMBINANT PROTEOLYTIC TYPTASES, ACTIVE SITE MUTANTS
               THEREOF, AND METHODS OF MAKING SAME
     10 <130> FILE REFERENCE: CIP TRYPTASE
C--> 12 <140> CURRENT APPLICATION NUMBER: US/09/598,982
C--> 13 <141> CURRENT FILING DATE: 2000-06-21
     15 <150> PRIOR APPLICATION NUMBER: 09/079,970
     16 <151> PRIOR FILING DATE: 1998-04-15
     18 <160> NUMBER OF SEQ ID NOS: 51
     20 <170> SOFTWARE: PatentIn Ver. 2.0
     22 <210> SEQ ID NO: 1
     23 <211> LENGTH: 735
     24 <212> TYPE: DNA
     25 <213> ORGANISM: Homo sapiens
     27 <220> FEATURE:
     28 <221> NAME/KEY: CDS
     29 <222> LOCATION: (1)..(735)
      31 <400> SEQUENCE: 1
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     33 Ile Val Gly Gly Gln Glu Ala Pro Arg Ser Lys Trp Pro Trp Gln Val 34 1 5 10 15
      36 agc ctg aga gtc cac ggc cca tac tgg atg cac ttc tgc ggg ggc tcc
     37 Ser Leu Arg Val His Gly Pro Tyr Trp Met His Phe Cys Gly Gly Ser
38 20 25 30
     40 ctc atc cac ccc cag tgg gtg ctg acc gca gcg cac tgc gtg gga ccg 41 Leu Ile His Pro Gln Trp Val Leu Thr Ala Ala His Cys Val Gly Pro
                                                                                        144
                                           40
                  35
     44 gac gtc aag gat ctg gcc gcc ctc agg gtg caa ctg cgg gag cag cac 45 Asp Val Lys Asp Leu Ala Ala Leu Arg Val Gln Leu Arg Glu Gln His 46 \phantom{0}50 \phantom{0}55 \phantom{0}60
      48 ctc tac tac cag gac cag ctg ctg ccg gtc agc agg atc atc gtg cac
      49 Leu Tyr Tyr Gln Asp Gln Leu Leu Pro Val Ser Arg Ile Ile Val His
                                70
                                                        75
     52 cca cag ttc tac acc gcc cag atc gga gcg gac atc gcc ctg ctg gag
53 Pro Gln Phe Tyr Thr Ala Gln Ile Gly Ala Asp Ile Ala Leu Leu Glu
                            85
                                                     90
     56 ctg gag gag ccg gtg aac gtc tcc agc cac gtc cac acg gtc acc ctg
57 Leu Glu Glu Pro Val Asn Val Ser Ser His Val His Thr Val Thr Leu
                    100
                                             105
                                                                      110
     60 ccc cct gcc tca gag acc ttc ccc ccg ggg atg ccg tgc tgg gtc act
      61 Pro Pro Ala Ser Glu Thr Phe Pro Pro Gly Met Pro Cys Trp Val Thr
                115
                                        120
                                                                 125
      64 ggc tgg ggc gat gtg gac aat gat gag cgc ctc cca ccg cca ttt cct
      65 Gly Trp Gly Asp Val Asp Asn Asp Glu Arg Leu Pro Pro Pro Phe Pro
                                     135
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68 ctg aag cag gtg aag gtc ccc ata atg gaa aac cac att tgt gac gca
69 Leu Lys Gln Val Lys Val Pro Ile Met Glu Asn His Ile Cys Asp Ala
                                                 155
70 145
                          150
72 aaa tac cac ctt ggc gcc tac acg gga gac gac gtc cgc atc gtc cgt
73 Lys Tyr His Leu Gly Ala Tyr Thr Gly Asp Asp Val Arg Ile Val Arg
                                                                                        528
                    165
                                               170
76 gac gac atg ctg tgt gcc ggg aac acc cgg agg gac tca tgc cag ggc
77 Asp Asp Met Leu Cys Ala Gly Asn Thr Arg Arg Asp Ser Cys Gln Gly 78 180 180
80 gac tee gga ggg eee etg gtg tge aag gtg aat gge aee tgg etg eag
                                                                                         624
81 Asp Ser Gly Gly Pro Leu Val Cys Lys Val Asn Gly Thr Trp Leu Gln
82 195 200 205
84 gcg ggc gtg gtc agc tgg ggc gag ggc tgt gcc cag ccc aac cgg cct
85 Ala Gly Val Val Ser Trp Gly Glu Gly Cys Ala Gln Pro Asn Arg Pro
86 210 215 220
88 ggc atc tac acc cgt gtc acc tac tac ttg gac tgg atc cac cac tat
89 Gly Ile Tyr Thr Arg Val Thr Tyr Tyr Leu Asp Trp Ile His His Tyr
                                                                                        720
90 225
                  230
                                                      235
                                                                                        735
92 gtc ccc aaa aag ccg
93 Val Pro Lys Lys Pro
                      245
97 <210> SEQ ID NO: 2
98 <211> LENGTH: 245
99 <212> TYPE: PRT
100 <213> ORGANISM: Homo sapiens
102 <400> SEQUENCE: 2
103 Ile Val Gly Gly Glu Ala Pro Arg Ser Lys Trp Pro Trp Gln Val 104 1 5 10 15
106 Ser Leu Arg Val His Gly Pro Tyr Trp Met His Phe Cys Gly Gly Ser 107 \phantom{\bigg|}20\phantom{\bigg|}25\phantom{\bigg|}30\phantom{\bigg|}
109 Leu Ile His Pro Gln Trp Val Leu Thr Ala Ala His Cys Val Gly Pro 110 35 40 45
112 Asp Val Lys Asp Leu Ala Ala Leu Arg Val Gln Leu Arg Glu Gln His
113 50 60
115 Leu Tyr Tyr Gln Asp Gln Leu Leu Pro Val Ser Arg Ile Ile Val His
116 65 70 75 80
118 Pro Gln Phe Tyr Thr Ala Gln Ile Gly Ala Asp Ile Ala Leu Leu Glu
119 85 90 95
121 Leu Glu Glu Pro Val Asn Val Ser Ser His Val His Thr Val Thr Leu
122 100 105 110
124 Pro Pro Ala Ser Glu Thr Phe Pro Pro Gly Met Pro Cys Trp Val Thr 125 115 120 125
127 Gly Trp Gly Asp Val Asp Asn Asp Glu Arg Leu Pro Pro Pro Pro Pro 128 130 135 140
130 Leu Lys Gln Val Lys Val Pro Ile Met Glu Asn His Ile Cys Asp Ala
131 145 150 155 160
133 Lys Tyr His Leu Gly Ala Tyr Thr Gly Asp Asp Val Arg Ile Val Arg
134 165 170 175
136 Asp Asp Met Leu Cys Ala Gly Asn Thr Arg Arg Asp Ser Cys Gln Gly
```

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180
                                         185
139 Asp Ser Gly Gly Pro Leu Val Cys Lys Val Asn Gly Thr Trp Leu Gln 140 195 200 205
142 Ala Gly Val Val Ser Trp Gly Glu Gly Cys Ala Gln Pro Asn Arg Pro 143 210 215 220
145 Gly Ile Tyr Thr Arg Val Thr Tyr Tyr Leu Asp Trp Ile His His Tyr 146 225 230 235 240
148 Val Pro Lys Lys Pro
149 245
149
152 <210> SEQ ID NO: 3
153 <211> LENGTH: 40
154 <212> TYPE: DNA
155 <213> ORGANISM: Artificial Sequence
157 <220> FEATURE:
158 <223> OTHER INFORMATION: Description of Artificial Sequence: PCR Primer
160 <400> SEQUENCE: 3
161 gggcccctcg agaaaagaat cgtcgggggt caggaggccc
164 <210> SEQ ID NO: 4
165 <211> LENGTH: 40
166 <212> TYPE: DNA
167 <213> ORGANISM: Artificial Sequence
169 <220> FEATURE:
170 <223> OTHER INFORMATION: Description of Artificial Sequence: PCR Primer
172 <400> SEQUENCE: 4
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176 <210> SEQ ID NO: 5
177 <211> LENGTH: 771
178 <212> TYPE: DNA
179 <213> ORGANISM: Homo sapiens
181 <220> FEATURE:
182 <221> NAME/KEY: CDS
183 <222> LOCATION: (7)..(753)
185 <400> SEQUENCE: 5
186 gggccc ctc gag aaa aga atc gtc ggg ggt cag gag gcc ccc agg agc
187 Leu Glu Lys Arg Ile Val Gly Gly Gln Glu Ala Pro Arg Ser
188
                               5
             1
                                                      10
190 aag tgg ccc tgg cag gtg agc ctg aga gtc cac ggc cca tac tgg atg
191 Lys Trp Pro Trp Gln Val Ser Leu Arg Val His Gly Pro Tyr Trp Met 192 15 20 25 30
194 cac ttc tgc ggg ggc tcc ctc atc cac ccc cag tgg gtg ctg acc gca
                                                                                144
195 His Phe Cys Gly Gly Ser Leu Ile His Pro Gln Trp Val Leu Thr Ala
196
                                              40
198 gcg cac tgc gtg gga ccg gac gtc aag gat ctg gcc gcc ctc agg gtg
                                                                                192
199 Ala His Cys Val Gly Pro Asp Val Lys Asp Leu Ala Ala Leu Arg Val
                 50
                                       55
202 caa ctg cgg gag cag cac ctc tac tac cag gac cag ctg ctg ccg gtc 203 Gln Leu Arg Glu Gln His Leu Tyr Tyr Gln Asp Gln Leu Leu Pro Val 65 70 75
                                                                                 240
206 age agg ate ate gtg cae eea eag tte tae ace gee eag ate gga geg
```

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210 gac atc gcc ctg ctg gag ctg gag gag ccg gtg aac gtc tcc agc cac 211 Asp Ile Ala Leu Leu Glu Leu Glu Glu Pro Val Asn Val Ser Ser His 212 95 100 100 105 110
214 gtc cac acg gtc acc ctg ccc cct gcc tca gag acc ttc ccc ccg ggg
                                                                                             384
215 Val His Thr Val Thr Leu Pro Pro Ala Ser Glu Thr Phe Pro Pro Gly 216 115 120 125
218 atg ccg tgc tgg gtc act ggc tgg ggc gat gtg gac aat gat gag cgc
219 Met Pro Cys Trp Val Thr Gly Trp Gly Asp Val Asp Asn Asp Glu Arg
220 130 135 140
222 ctc cca ccg cca ttt cct ctg aag cag gtg aag gtc ccc ata atg gaa
223 Leu Pro Pro Pro Phe Pro Leu Lys Gln Val Lys Val Pro Ile Met Glu
224 145 150 155
226 aac cac att tgt gac gca aaa tac cac ctt ggc gcc tac acg gga gac
                                                                                             528
227 Asn His Ile Cys Asp Ala Lys Tyr His Leu Gly Ala Tyr Thr Gly Asp
228 160 165 170
230 gac gtc cgc atc gtc cgt gac gac atg ctg tgt gcc ggg aac acc cgg
231 Asp Val Arg Ile Val Arg Asp Asp Met Leu Cys Ala Gly Asn Thr Arg
232 175 180 185 190
234 agg gac toa tgc cag ggc gac tec gga ggg ccc etg gtg tgc aag gtg
235 Arg Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys Lys Val
236 195 200 205
238 aat ggc acc tgg ctg cag gcg ggc gtg gtc agc tgg ggc gag ggc tgt
239 Asn Gly Thr Trp Leu Gln Ala Gly Val Val Ser Trp Gly Glu Gly Cys
240 210 215 220
242 gcc cag ccc aac cgg cct ggc atc tac acc cgt gtc acc tac tac ttg 243 Ala Gln Pro Asn Arg Pro Gly Ile Tyr Thr Arg Val Thr Tyr Tyr Leu 244 225 230 230 235 \phantom{\bigg|}
                                                                                             771
246 gac tgg atc cac cac tat gtc ccc aaa aag ccg tgaagcggcc gccgtcgt
247 Asp Trp Ile His His Tyr Val Pro Lys Lys Pro 248 240 245
251 <210> SEQ ID NO: 6
252 <211> LENGTH: 249
253 <212> TYPE: PRT
254 <213> ORGANISM: Homo sapiens
256 <400> SEQUENCE: 6
257 Leu Glu Lys Arg Ile Val Gly Gly Gln Glu Ala Pro Arg Ser Lys Trp 258 \phantom{-}1\phantom{+}5\phantom{+}5\phantom{+}10\phantom{+}15\phantom{+}
260 Pro Trp Gln Val Ser Leu Arg Val His Gly Pro Tyr Trp Met His Phe 261 20 25 30
263 Cys Gly Gly Ser Leu Ile His Pro Gln Trp Val Leu Thr Ala Ala His 264 35 40 45
266 Cys Val Gly Pro Asp Val Lys Asp Leu Ala Ala Leu Arg Val Gln Leu
267 50 60
269 Arg Glu Gln His Leu Tyr Tyr Gln Asp Gln Leu Leu Pro Val Ser Arg
270 65 70 75 80
272 Ile Ile Val His Pro Gln Phe Tyr Thr Ala Gln Ile Gly Ala Asp Ile
```

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Input Set : A:\Tryptase.app
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275 Ala Leu Leu Glu Leu Glu Glu Pro Val Asn Val Ser Ser His Val His 100 105 276 278 Thr Val Thr Leu Pro Pro Ala Ser Glu Thr Phe Pro Pro Gly Met Pro 279 115 120 125 281 Cys Trp Val Thr Gly Trp Gly Asp Val Asp Asn Asp Glu Arg Leu Pro 282 130 135 140 284 Pro Pro Phe Pro Leu Lys Gln Val Lys Val Pro Ile Met Glu Asn His 285 145 150 150 155 160 287 Ile Cys Asp Ala Lys Tyr His Leu Gly Ala Tyr Thr Gly Asp Asp Val 288 165 170 175 290 Arg Ile Val Arg Asp Asp Met Leu Cys Ala Gly Asn Thr Arg Arg Asp 291 180 185 190 293 Ser Cys Gln Giy Asp Ser Gly Gly Pro Leu Val Cys Lys Val Asn Gly 294 195 200 205 296 Thr Trp Leu Gln Ala Gly Val Val Ser Trp Gly Glu Gly Cys Ala Gln 297 210 215 220 299 Pro Asn Arg Pro Gly Ile Tyr Thr Arg Val Thr Tyr Tyr Leu Asp Trp 300 225 230 235 240 302 Ile His His Tyr Val Pro Lys Lys Pro 303 306 <210> SEQ ID NO: 7 307 <211> LENGTH: 27 308 <212> TYPE: DNA 309 <213> ORGANISM: Artificial Sequence 311 <220> FEATURE: 312 <223> OTHER INFORMATION: Description of Artificial Sequence: Mutagenesis 313 Oligo 315 <400> SEQUENCE: 7 27 316 gaggagccgg tgaaggtctc cagccac 319 <210> SEQ ID NO: 8 320 <211> LENGTH: 771 321 <212> TYPE: DNA 322 <213> ORGANISM: Homo sapiens 324 <220> FEATURE: 325 <221> NAME/KEY: CDS 326 <222> LOCATION: (7)..(753) 328 <400> SEQUENCE: 8 329 gggccc ctc gag aaa aga atc gtc ggg ggt cag gag gcc ccc agg agc 330 Leu Glu Lys Arg Ile Val Gly Gly Gln Glu Ala Pro Arg Ser 331 1 5 10 333 aag tgg ccc tgg cag gtg agc ctg aga gtc cac ggc cca tac tgg atg 96 334 Lys Trp Pro Trp Gln Val Ser Leu Arg Val His Gly Pro Tyr Trp Met 335 15 20 25 30 335 15 337 cac ttc tgc ggg ggc tcc ctc atc cac ccc cag tgg gtg ctg acc gca 144 338 His Phe Cys Gly Gly Ser Leu Ile His Pro Gln Trp Val Leu Thr Ala 339 35 40 45 341 gcg cac tgc gtg gga ccg gac gtc aag gat ctg gcc gcc ctc agg gtg 192 342 Ala His Cys Val Gly Pro Asp Val Lys Asp Leu Ala Ala Leu Arg Val

VERIFICATION SUMMARY

DATE: 07/05/2000

PATENT APPLICATION: US/09/598,982

TIME: 18:43:08

Input Set : A:\Tryptase.app
Output Set: N:\CRF3\07052000\I598982.raw

 $\ \, \text{L:12 M:270 C: Current Application Number differs, Replaced Application Number L:13 M:271 C: Current Filing Date differs, Replaced Current Filing Date }$